# Assessment of Microplastic Contaminations in African Catfish (*Clarias gariepinus*) From Selected Fish Farms in Awka Metropolis, Anambra State, Nigeria

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### Abstract

This study assessed microplastic contaminations in African catfish (Clarias gariepinus) and water samples from selected fish farms in Awka Metropolis, Anambra State, Nigeria. It also examined the microplastic abundance, particle length, color, morphology, and polymer composition in different fish tissues (gills, intestines, and muscle) and water samples. Microplastic extraction was done using density separation techniques, and polymer identification was conducted using Fourier Transform Infrared (FTIR) spectroscopy. The study also analyzed the physico-chemical parameters of water, including temperature, pH, biological oxygen demand (BOD), and dissolved oxygen (DO). Samples were collected from three commercial fish farms, designated as PF (Farm 1), UF (Farm 2), and HF (Farm 3). The data generated from the study were subjected to statistical analysis. Results obtained indicated significant (P < 0.05) microplastic contaminations in all examined fish tissues, with the highest levels found in HF. Specifically, UF's gills showed the highest microplastic abundance (1.23  $\pm$ 0.05 particles/g), while PF had the lowest (0.40  $\pm$  0.05 particles/g). In the intestines, HF exhibited the highest concentration  $(1.14 \pm 0.05 \text{ particles/g})$ , while PF had lower levels (0.71) $\pm 0.03$  particles/g). Water samples also revealed significant contamination, with HF having the highest concentration  $(1.37 \pm 0.04 \text{ particles/L})$ , surpassing UF  $(0.79 \pm 0.09 \text{ particles/L})$  and *PF*  $(0.37 \pm 0.04 \text{ particles/L})$ . *Physico-chemical analysis showed significant difference* (p < 0.05)in environmental conditions among the farms, particularly with phosphate levels being highest in HF (15.58  $\pm$  0.50 mg/L) compared to UF (9.43  $\pm$  0.33 mg/L) and PF (8.48  $\pm$  0.24 mg/L). Other parameters, including temperature and dissolved oxygen, varied notably, with HF often displaying the most extreme values. The presence of microplastics in muscle tissues raises potential health risks for human consumers. The study concludes that microplastic contamination in fish and water samples poses a critical environmental and public health issue. It is recommended that constant monitoring of fish feed and water sources. Also, proper waste management practices should be carried out on a regular basis.

Keywords: Microplastics, Fish, Water quality, Aquatic pollution

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### INTRODUCTION

Microplastics (MPs) are a global issue because they are released all over the world [1]. Different plastic goods have become commonplace in people's daily lives. Plastics' use has expanded 25-fold in the previous 40 years owing to minimal cost, durability, low weight, and elasticity [2].Worldwide, plastics are widely utilized in food packaging, building and construction, automobile items, electrical devices, domestic sports and recreational, farming, healthcare, and plastic furnishings. MPs are microscopic plastic grains that are said to be common in discarded plastic fragment goods [3]. Primary MPs are produced small plastic granules to be used in facial-cleansers and cosmetics, air blasting technology, and vectors for drugs in medicine, while secondary nano plastics are tiny plastic remnants deteriorated from MPs debris [4]. The most prevalent waste materials are brought to the seas by rivers, floods, and winds that pollute the ocean and beaches ecosystem. Discarded fishing craft, plastic bags, food containers, and plastic drinks bottles (water and cold drinks) pollute the water ecosystem [5]. Mishandling of enormous anthropogenic activities could introduce many xenobiotic pollutants to water environments around the planet, either deliberately or accidentally [6]. MPs are reported to be present at all levels of aquatic environments, posing threat to major biota [7]. MPs have been found in edible fish, according to various research, and as a result of biomagnifications, MPs penetrate human systems [8]. MP-induced impairments in species ranged from minimal biological systems disturbance to substantial unfavorable consequences that resulted in mortality [9]. Physiological harm as a result of MPs accumulating within the digestive system; disruption of organisms' energy flow as a result of MPs expelling as pseudofeces; and inner body tissue exposed to MPs after transfer within the body were all designated as harmful by Ma et al. [10]. They also serve as a pathway for organic contaminants and trace metals to reach aquatic habitats [11]. MPs can affect predatory behavior in fish and cause misunderstanding between MPs and genuine prey [12], leading to malnutrition and MP storage in key organs such the gills, gut, and stomach [13]. MPs were also found in fish muscle/meat, which is mainly consumed by humans [14]. Growth retardation, hormone disruption, metabolic perturbation, oxidative stress, immunological and neurotoxicity malfunction, and genotoxicity behavioural alterations are all caused by a buildup of MPs [15]. The ingestion of microplastics by fish occurs through various mechanisms, including filter feeding and ingestion of contaminated feed and prey [16]. Once ingested, microplastics can accumulate in the digestive tract of fish, potentially transferring toxic chemicals and additives associated with plastics into their tissues [17]. Studies indicated that these contaminants may pose health risks to humans when fish containing microplastics are consumed, although the extent of these risks requires further investigation [18]. Furthermore, microplastics have the potential to act as vectors for pathogens and pollutants in aquatic ecosystems, which can further complicate their impact on public health [18]. The global scale of microplastic pollution underscores the urgency of addressing this issue through interdisciplinary research and effective environmental management strategies [19]. The presence of microplastics in fish represents a multifaceted challenge with implications for both environmental sustainability and public health. Addressing this issue requires concerted efforts to mitigate plastic pollution at its source, enhance monitoring and detection methods, and further elucidate the potential health risks associated with microplastic ingestion through seafood consumption[20].

Research on microplastics in fish and their potential impact on human health is motivated by the urgent need to address pervasive contamination of aquatic ecosystems. Microplastics, originating from various sources including plastic debris breakdown and microbeads, pose significant risks to marine life [21]. Fish are particularly vulnerable as they ingest these particles, which can accumulate in their tissues over time [22]. This accumulation may lead to the biomagnification of contaminants associated with microplastics, potentially increasing exposure risks for humans who consume seafood [23]. The transfer of microplastics and associated chemicals from fish to humans through seafood consumption highlights critical health concerns [24]. These particles have the capacity to leach toxic substances into tissues, potentially leading to adverse health effects such as inflammation and oxidative stress [25]. Scientific research plays a vital role in elucidating the mechanisms of microplastic uptake, accumulation, and toxicity in both marine organisms and humans [26]. This research is essential for informing risk assessments and creating awareness on the health implications associated with microplastics contamination [27], studying microplastics in fish and their implications for human health is crucial for understanding the extent of contamination, assessing associated health risks, and informing policies aimed at reducing plastic pollution globally. The primary aim of this research is to assess the presence and extent of microplastic contamination in the tissues of Clarias gariepinus and their surrounding aquatic environment across the selected commercial fish farms.

#### METHODOLOGY

#### **Study Area**

The study area is within Awka metropolis located in the Eastern part of Nigeria, Awka is defined by a wide variety of culture and markets where everything is sold from basic food stuffs to household items, commercial fish farming is also practiced in Awka which makes it a good area for the study of Microplastics in aquatic environments. Three commercial fish farms were selected for this research, based on their geographical location, scale of operations, and management practices namely; (UF), (HF) and (PF). These farms are located in distinct areas to allow for comparative analysis of microplastic contamination. Each farm practices intensive aquaculture, cultivating *Clarias gariepinus* under similar environmental conditions but vary in terms of water sources, feed types, and waste management.

### Sample collection

A total of three (3) adult *Clarias gariepinus* were randomly sampled from each fish farm, making a total of Nine (9) catfish across all farms. From each farm, fish of uniform size and weight were selected to ensure consistency in the microplastic analysis. Three different Water samples were collected each for Microplastic and physicochemical analysis, from each fish farm pond to ensure representative sampling making it a total of 18 samples across the three farms. The samples were stored in pre-cleaned glass bottles and immediately transported to Alpha laboratory Alpha Research Laboratory located at 25 Ezeudu street off Zik Avenue Awka, Anambra for further analysis.

### Microplastic extraction protocol

Extraction was carried out using a two-step optimized microplastic extraction procedure, including chemical digestion and density separation. With this focus, the extraction protocol was adopted from Karami *et al.*, 2017). 20 gm of each was taken into a 250-ml conical flask. After that, 100 ml of a 10% KOH solution (1:10 w/v) was added to it and sealed for 96 h (4 days) under a laminar hood to complete the digestion. This solution was then separated and poured into another conical flask. 40 ml of saturated NaCl solution were then added to the solution and kept for 24 h (1 day) for density separation. Bone and flesh materials including the microplastics particles of fish feeds could be compromised by acidic digestion. Therefore, KOH digestion protocol was applied that might reduce the spectroscopic difficulty in

identifying the polymeric composition. Again, the fish sample is composed of complex biotic materials. Saline solution, e.g., NaCl, is denser than water and causes plastic materials to float to the surface. The supernatant was then vacuum filtered through 0.45  $\mu$ m Whatman glass microfiber filter paper (GF/F Whatman TM, USA). These filter papers were then kept in Petri dishes, dried, and preserved for visual and polymeric inspection.

### Visualization of microplastics by stereomicroscope

The conventional approach for identifying microplastics involves utilizing stereo-microscopy to visually detect them based on their size, shape, and color. In this study, all the filter papers were visualized by Leica EZ4E stereomicroscope (Leica, Germany) with 16x, 20x, 30x and 35x zoom on the basis of needs. The characterization of microplastics utilizing this technology primarily relies on their morphological and physical attributes, contingent upon the specific research objectives. The size categories of polymer species exhibit variation, spanning a range of 1–5 mm. This methodology does not furnish data regarding the specific identity of the polymer. Nevertheless, in order to assess the presence of potential plastic fragments, image processing software such as digital image J software was utilized to quantitatively determine the prevalence of microplastics. Microscopy is a process that may be influenced by subjectivity, tedium, and reliance on the observer. However, the utilization of automation and signal processing through image J software has the potential to mitigate these limitations. Nevertheless, this analytical technique lacked the ability to effectively differentiate microplastic particles from other anthropogenic synthetic particles. Therefore, in order to validate the existence of microplastic polymers, we further employed additional techniques such as FTIR spectroscopic analysis.

### **Polymeric verification using FTIR**

FTIR (Fourier Transform Infrared Spectrophotometer, Model no. IR Prestige-21, SHIMADZU, Japan) was utilized to validate the polymeric kinds of suspect microplastics. A representative number of samples were selected for characterization of microplastics by the FTIR. These microplastics were deemed to be indicative of the most often observed types of particles across all samples. Microplastics were evenly dispersed throughout a KBr crystal disc. Spectra were captured as the mean of 64 scans in the 4000-400 cm-1 spectral wave region at a resolution of 4cm-1. Each sample spectrum was verified by database from John Wiley & Sons, Inc.'s online spectral repository as well as by Jung *et al.* [28].

### **Quality control**

Concerted efforts were taken to reduce and eliminate airborne contamination during laboratory screening for MPs. Before starting, all glassware items were washed with mild detergent and thoroughly rinsed in running borehole water. Washed bottles and flasks were then autoclaved, clad in aluminium foil and stored in cupboards. All work surfaces were wiped down with 70% ethanol solution before the start of procedures. Specimen dissection, organ removals, decanting of KOH aliquots for digestion etc. were done in fume chambers with vacuum suction pumps. All persons involved wore cotton lab coats and latex (rubber) disposable gloves, Laboratory doors and windows were also shut, to reduce wind-borne contamination.

### Physicochemical analysis of water samples

### **Determination of PH value**

The pH of the water samples was determined using a pH universal designed to determine the pH of solutions over a wide range of values. 10ml of each water sample was measured into 100 cm3 beaker and 2 drops of solution of the universal pH indicator was added. The colour developed on the samples is matched with the standard pH colour chart [29]. The colours from

yellow to red indicate an acidic solution, colours blue to violet indicate an alkaline solution and a green colour indicates that a solution is neutral

# **Determination of temperature**

The temperature of all the water samples was determined using a simple mercury-in-glass thermometer calibrated in degrees centigrade as described by Edema *et al.* [30] and Dinrifo *et al.*[31].

### **Determination of nitrates**

50ml of each water sample was measured. 1g of sodium sulphate was added and shaken thoroughly. 5ml was measured from the mixture in a separate test tube. 1ml of ferric chloride indicator was added with 10mls of concentrated  $H_2SO_4$ . The remaining 45ml solution was mixed with the sample and allowed to develop for about 30mins to 1hr. The absorbance was read with the aid of spectrometer machine at 490nm.

### **Determination of phosphate**

The phosphate contents of the water were measured by colorimetric method, according to Golterman *et al.* (1978). 25ml of the sample was measured in a 250ml of beaker. 3ml of barium chloride solution was added and stirred then allowed to stay for 30 minutes before being taken to Corning colorimeter 253 for determination of phosphate at 710nm wavelength, respectively. Blank solutions were prepared in the same manner, then the disposable cuvette was filled and reading was taken on the colorimeter. The concentrations of phosphate were determined by extrapolating from the calibration curve.

PO4 (mg/l) =Mass of PO4 read from curve x 1000/ ml sample.

### **Determination of Nitrite**

One hundred (100) ml of water sample was poured into a crucible, evaporated to dryness, and cooled. 2ml of phenoldisulphonic acid was added and smeared around the crucible, after 10minutes, 10ml of distilled water was added followed by 5ml of strong ammonia solution. Setting the spectrophotometer at the wave length of 430nm, absorbance of the sample treated was obtained, using distilled water as blank. The concentration of nitrate-nitrogen was obtained from the Calibration curve in mgL-1 as described by APHA [32].

### Dissolved ammonia

Dissolved ammonia in pond water was measured by distillation method. 300ml of pond water was placed in a flat bottom flask. Excess amount of KOH (18M, 20.0ml) was added to it, and then the flask was heated and distillate. Liberated soluble ammonia with water was collected in the conical flask. The 10.0 ml of above solution was pipetted out into the titration flask and it was titrated with 0.01 M HCl using methyl orange as an indicator [33].

NH3 (mg/l) = <u>Titre value  $\times$  100</u>

### Sample volume used

### Chemical oxygen demand

The COD of water sample is a measure of the oxidizable organic matters present in the sample. 10.0 ml of KMnO4 solution (0.0125M) was added into a stoppered bottle containing 100.0 ml of pond water, then 10.0 ml of H2SO4 (2.5M) was added to it. The same reagents were added to 100ml of distilled water (As a control). Both the samples and control were placed in a boiling water bath for 30 minutes, then removed and allowed to cool. 3.0 ml of KI Solution (0.3M) was added to it and shaken well. 10.0 ml of the resultant solution was pipetted out and titrated with standard Na2S2O3 (0.0125M) solution until only a faint yellow colour remained. A few drops of starch indicator were added and the titration was continued until the blue colour is just discharged as described by APHA [32].

### **Biological oxygen demand**

A 10ml aliquot of water sample diluted in 90ml distilled water and dispensed into BOD volumetric flask was added a mixture of 22.5g/l MgSO4.7H2O and 2ml alkali-iodide-azide reagent. 2ml of CaCl2 and FeCl3.6H2O were also added in the same manner. The sample was mixed by inverting the BOD flask many times producing a brownish cloudy solution indicating presence of oxygen. A brown precipitate which settles at the bottom of the flask was dissolved by the addition of 2ml of concentrated H2S04. The flask was kept in the incubator for 5 days and 50ml of the water sample titrated against 0.025N sodium thiosulphate to a pale yellow color. Addition of 2ml of starch solution to the titrate gives a blue color and the titration continued until a clear solution was formed. Concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used as described by APHA [32].

The dissolved oxygen is calculated from the formula:

 $DO1 = (f x titrant volume \times 8000) / Volume of sample$ 

BOD = (DO1 - DO5) / 0.05

Where, DO1 is dissolved oxygen for day 1; DO5 is dissolved oxygen for day 5

### **3.5** Statistical analysis

The result from the laboratory analysis of microplastic contamination in fish and water samples was subjected to one way analysis of variance (ANOVA) and differences in means was separated using Duncan multiple Range Test at 5% significance level. The statistical analysis was done using R-statistical software (R-2023).

### RESULTS

Table 1 presents the abundance of microplastics found in the gills, intestine, muscle, and water samples from the three selected fish farms: PF (Farm 1), UF (Farm 2), and HF (Farm 3). The data are expressed as mean  $\pm$  standard error, and statistical significance was determined using ANOVA with superscripts indicating significant differences (P < 0.05). Microplastic abundance varied significantly across fish farms and tissues. The highest concentration of microplastics was observed in the gills of fish from UF (1.23  $\pm$  0.05 particles/g), while the lowest concentration was found in the gills of PF (0.40  $\pm$  0.05 particles/g). In the intestine, HF showed the highest accumulation (1.14  $\pm$  0.05 particles/g), whereas PF had the lowest (0.71  $\pm$  0.04 particles/g), whereas UF exhibited the lowest (0.21  $\pm$  0.06 particles/g). Water samples followed a similar trend, with HF showing the highest microplastic concentration (1.37  $\pm$  0.04 particles/L). In summary, microplastic accumulation in both fish tissues and water was significantly higher in HF compared to UF and PF, with notable differences in contamination levels among the farms.

Table 1: Microplastics Abundance in Micro	plastics in Fish and Water Samples from
Different Farms	

Parameters	PF (Farm 1)	UF (Farm 2)	HF (Farm 3)
Gill	$0.40{\pm}0.05^{\circ}$	$1.23\pm0.05^{\rm a}$	$0.76\pm0.07^{\rm b}$
Intestine	$0.71 \pm 0.03^{\circ}$	$0.93\pm0.06^{\text{b}}$	$1.14\pm0.05^{\rm a}$
Muscle	$0.51\pm0.05^{\circ}$	$0.21\pm0.06^{\text{b}}$	$0.54{\pm}0.05^{\rm a}$
Water	$0.37\pm0.04^{\text{b}}$	$0.79\pm0.09^{\rm c}$	$1.37\pm0.04^{\rm a}$

Means with the same superscript within rows are not significantly different (P>0.05)

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#### Length variations of micro plastics in fish samples

The average length of microplastics found in the gills, intestines, muscle, and water samples from fish collected from three different fish farms (PF, UF, HF) are presented in Table 2. The lengths are measured in micrometers ( $\mu$ m). The results show that the water samples generally had the longest microplastic particles, especially in PF (1654.66  $\mu$ m) and HF (1692  $\mu$ m). Among the fish tissues, the intestine in UF exhibited the longest average microplastic length (1410.66  $\mu$ m), while the muscle consistently showed smaller particles compared to the other tissues, except in HF where the muscle had a relatively high average length (806  $\mu$ m). The gills showed the shortest microplastic particles in all treatments, particularly in PF (417.33  $\mu$ m).

 Table 2: Length variations of Microplastics in Fish and Water Samples from Different

 Farms

Parameters	PF (Farm 1)	UF (Farm 2)	HF (Farm 3)
Gill	$417.33\pm2.08^{\rm c}$	$526.33 \pm 3.05^{b}$	$814.34\pm2.08^{a}$
Intestine	$744.33 \pm 1.50^{\mathrm{a}}$	$1410.66 \pm 92.08^{a}$	$1161 \pm 5.13^{b}$
Muscle	$511.33 \pm 2.51^{\circ}$	$746.66 \pm 3.51^{b}$	$806.00\pm2.00^{\mathrm{a}}$
Water	$1654.66 \pm 2.51^{b}$	$1211.66 \pm 3.51^{\circ}$	$1692.00 \pm 3.00^{\rm a}$

Means with the same superscript within rows are not significantly different (P>0.05) Colour variations of Microplastics in fish samples

Analysis of color variations in microplastics (MPs) across different fish samples (intestine, gill, muscle) and water samples from three aquaculture farms (PF, UF, HF) revealed significant differences in the abundance of various colored microplastics, as Presented in Table 3. In the intestine samples, the HF farm had the highest abundance of blue microplastics ( $33.33 \pm 3.51$ ), significantly greater than the PF ( $16.38 \pm 0.31$ ) and UF ( $24.00 \pm 2.64$ ) farms (P < 0.05). Brown microplastics were also most abundant in the HF farm ( $45.00 \pm 4.00$ ), while the UF farm exhibited the lowest abundance ( $10.16 \pm 0.32$ ). Notably, red microplastics were found exclusively in the UF ( $10.37 \pm 0.17$ ) and HF ( $11.66 \pm 3.05$ ) farms, with none detected in the PF farm. White microplastics were more prevalent in the UF farm ( $53.33 \pm 3.51$ ), followed by PF ( $33.33 \pm 3.05$ ), and least in HF ( $30.42 \pm 0.31$ ). The gill samples showed no significant differences in blue microplastics across farms, with the highest values found in UF ( $39.33 \pm 3.05$ ) and PF ( $25.99 \pm 0.75$ ). The HF farm presented lower brown microplastics were minimal in all samples, with no significant presence. White microplastics were minimal in all samples, with no significant presence. White microplastics were motably abundant in HF ( $62.58 \pm 3.16$ ), followed closely by PF ( $44.33 \pm 2.51$ ) and UF ( $39.99 \pm 0.79$ ).

For muscle samples, blue microplastics were most abundant in PF ( $45.89 \pm 1.01$ ) and UF ( $40.16 \pm 1.75$ ), while HF had a lower count ( $28.04 \pm 0.77$ ). Brown microplastics were more prevalent in HF ( $34.19 \pm 2.02$ ) than in UF ( $8.80 \pm 1.16$ ). There were no detections of red or pink microplastics in any of the muscle samples.

In water samples, brown microplastics were most abundant in UF ( $62.75 \pm 2.30$ ) and PF ( $41.40 \pm 1.64$ ), while HF had a significantly lower count ( $10.66 \pm 1.52$ ). Blue microplastics were exclusively found in HF ( $21.85 \pm 1.77$ ), with no detection in PF. White microplastics were most abundant in PF ( $56.33 \pm 1.82$ ) and HF ( $62.13 \pm 1.99$ ), indicating significant variation in microplastic color composition across farms.

In general, the HF farm consistently showed a higher abundance of blue microplastics in the intestine and water samples, while PF demonstrated a varied color profile, indicating potential differences in environmental exposure and farming practices.

Farms			
Parameters	PF (Farm 1)	UF (Farm 2)	HF (Farm 3)
1. Intestine			
Blue	$16.38\pm0.31^{\circ}$	$24.00\pm2.64^{b}$	$33.33\pm3.51^{\mathrm{a}}$
Brown	$42.33\pm3.51^{\text{b}}$	$10.16 \pm 0.32^{\circ}$	$45.00\pm4.00^{\rm a}$
Red	$0.00\pm0.00^{\circ}$	$10.37\pm0.17^{b}$	$11.66\pm3.05^{\rm a}$
Pink	$11.75\pm0.29^{\mathrm{a}}$	$0.00\pm0.00^{ m c}$	$0.00\pm0.00^{ m c}$
White	$33.33\pm3.05^{\text{b}}$	$53.33\pm3.51^{\mathrm{a}}$	$30.42\pm0.31^b$
2. Gill			
Blue	$25.99\pm0.75^{\text{b}}$	$39.33 \pm 3.05a$	$19.89\pm0.84^{\rm c}$
Brown	$38.00\pm2.00^{\rm a}$	$8.33 \pm 1.52^{\rm c}$	$15.83\pm1.60^{\mathrm{b}}$
Red	$8.33\pm2.08^{\text{b}}$	$10.80\pm0.91^{a}$	$0.00\pm0.00$
Pink	$0.00\pm0.00$	$0.00\pm0.00$	$0.00\pm0.00$
White	$44.33\pm2.51^{\text{b}}$	$39.99\pm0.79^{\rm c}$	$62.58\pm3.16^{\rm a}$
3. Muscle			
Blue	$45.89 \pm 1.01^{\mathrm{a}}$	$40.16\pm1.75^{\mathrm{b}}$	$28.04\pm0.77^{\rm c}$
Brown	$20.62\pm0.54^{\text{b}}$	$8.80 \pm 1.16^{\circ}$	$34.19\pm2.02^{\rm a}$
Red	$0.00\pm0.00$	$0.00\pm0.00$	$0.00\pm0.00$
Pink	$0.00\pm0.00$	$0.00\pm0.00$	$0.00\pm0.00$
White	$35.05\pm0.16^{\circ}$	$51.00 \pm 1.90^{\rm a}$	$39.60\pm2.94^{b}$
4. Water			
Blue	$0.00\pm0.00$	$6.98\pm0.97^{b}$	$21.85 \pm 1.77^{a}$
Brown	$41.40 \pm 1.64^{b}$	$62.75\pm2.30^{\mathrm{a}}$	$10.66 \pm 1.52^{\circ}$
Red	$0.00\pm0.00$	$0.00\pm0.00$	$0.00\pm0.00$
Pink	$2.80\pm0.85^{\rm c}$	$4.32\pm1.15^{\text{b}}$	$8.54\pm0.50^{\rm a}$
White	$56.33 \pm 1.82^{\text{b}}$	$29.64 \pm 1.90^{\circ}$	$62.13\pm1.99^{a}$

Table 3: Colour Variations of Microplastics in Fish and Water Samples from Different Farms

Means with same superscript within rows are not significantly different (P > 0.05)

### Morphological variations in fish samples

The analysis of morphological variations across different fish samples (intestine, gill, muscle) and water samples from three aquaculture farms (PF, UF, HF) revealed significant differences in the abundance of various components, as presented in Table 4. In the intestine samples, the HF farm had the highest abundance of filament ( $32.91 \pm 2.54$ ), significantly greater than the PF ( $11.23 \pm 1.36$ ) and UF ( $20.17 \pm 1.36$ ) farms (P < 0.05). Pellet content was also most abundant in PF ( $50.32 \pm 1.17$ ), while UF ( $34.14 \pm 1.03$ ) and HF ( $38.22 \pm 2.03$ ) showed relatively lower values. Foam was more prevalent in UF ( $18.84 \pm 1.61$ ) compared to PF ( $11.76 \pm 1.59$ ) and HF ( $8.48 \pm 1.01$ ). Fiber was significantly higher in PF ( $28.63 \pm 1.18$ ) than HF ( $10.22 \pm 1.68$ ), with UF showing an intermediate level ( $25.06 \pm 1.10$ ). In the gill samples, UF showed the highest filament abundance ( $14.13 \pm 2.20$ ), significantly greater than HF and PF ( $0.00 \pm 0.00$  for both) (P < 0.05). Foam was detected only in PF ( $11.68 \pm 1.54$ ), with none observed in UF and HF. Fiber content was highest in UF ( $39.23 \pm 2.92$ ), followed by PF ( $28.87 \pm 1.19$ ) and HF ( $25.51 \pm 1.39$ ), showing significant differences across the farms.

For the muscle samples, UF again demonstrated the highest filament abundance ( $52.23 \pm 1.66$ ), which was significantly higher than PF (40.09  $\pm$  1.65) and HF (21.45  $\pm$  1.26) (P < 0.05). Film content was highest in HF ( $39.55 \pm 1.97$ ), followed by PF ( $31.22 \pm 1.07$ ) and UF ( $12.30 \pm 1.12$ ). Foam was only present in PF (14.19  $\pm$  1.14), showing a significant difference from the other farms. In the water samples, UF had the highest pellet content (59.80  $\pm$  2.70), which was significantly greater than HF ( $21.23 \pm 1.72$ ), with PF showing no detectable pellet. Foam was not observed in any of the water samples. Film was most abundant in HF (67.43  $\pm$  2.89), followed by UF ( $43.03 \pm 2.51$ ) and PF ( $79.48 \pm 1.83$ ), with significant differences in film across farms (P < 0.05). Overall, significant differences in the abundance of the various components were observed across the farms for all tissue types and water samples, indicating potential differences in environmental exposure and farming practices.

Different Farms	0	1	1
Parameters	PF (Farm 1)	UF (Farm 2)	HF (Farm 3)
1. Intestine			
Filament	$11.23 \pm 1.36^{\circ}$	$20.17\pm1.36^{\mathrm{b}}$	$32.91\pm2.54^{\rm a}$
Pellet	$50.32 \pm 1.17^{\mathrm{a}}$	$34.14 \pm 1.03^{\text{b}}$	$38.22\pm2.03^{\text{b}}$
Film	$0.00 \pm 0.00^{b}$	$0.00 \pm 0.00^{b}$	$13.09 \pm 1.31a$

Table 4: Morphological Variations of Microplastics in Fish and Water Samples from
Different Farms

Pellet	$50.32 \pm 1.17^{\rm a}$	$34.14 \pm 1.03^{\text{b}}$	$38.22\pm2.03^{\mathrm{b}}$
Film	$0.00\pm0.00^{\rm b}$	$0.00\pm0.00^{\mathrm{b}}$	$13.09 \pm 1.31^{\mathrm{a}}$
Foam	$11.76 \pm 1.59^{\mathrm{b}}$	$18.84 \pm 1.61^{a}$	$8.48 \pm 1.01^{\circ}$
Fiber	$28.63 \pm 1.18^{\rm a}$	$25.06 \pm 1.10^{\mathrm{b}}$	$10.22 \pm 1.68^{\circ}$
2 Gill			
Filament	$0.00\pm0.00^{\mathrm{a}}$	$14.13 \pm 2.20^{b}$	$0.00\pm0.00^{\mathrm{a}}$
Pellet	$40.66 \pm 1.52^{\mathrm{a}}$	$9.23 \pm 1.12^{\circ}$	$35.05 \pm 1.73^{\text{b}}$
Film	$22.78 \pm 1.76^{b}$	$41.24\pm1.13^{\rm a}$	$12.22\pm2.05^{\circ}$
Foam	$11.68 \pm 1.54^{\text{b}}$	$0.00\pm0.00^{\circ}$	$31.54 \pm 1.37^{\mathrm{a}}$
Fiber	$28.87 \pm 1.19^{\mathrm{b}}$	$39.23\pm2.92^{\rm a}$	$25.51 \pm 1.39^{\circ}$
3. Muscle			
Filament	$40.09\pm1.65^{\rm b}$	$52.23 \pm 1.66^{\mathrm{a}}$	$21.45 \pm 1.26^{\circ}$
Pellet	$0.00\pm0.00^{\mathrm{b}}$	$8.23 \pm 1.07^{\rm a}$	$0.00\pm0.00^{\rm b}$
Film	$31.22\pm1.07^{\mathrm{b}}$	$12.30 \pm 1.12^{\circ}$	$39.55 \pm 1.97^{\mathrm{a}}$
Foam	$14.19 \pm 1.14^{\mathrm{b}}$	$0.00\pm0.00^{\circ}$	$11.78 \pm 1.06^{a}$
Fiber	$16.47 \pm 1.36^{\circ}$	$29.02 \pm 1.07^{\rm a}$	$26.28\pm2.05^{\mathrm{b}}$
4 Water			
Filament	$13.53 \pm 1.50^{\mathrm{b}}$	$0.00\pm0.00^{\circ}$	$21.23 \pm 1.72^{\mathrm{a}}$
Pellet	$0.00\pm0.00^{\mathrm{b}}$	$59.80\pm2.70^{\mathrm{a}}$	$0.00\pm0.00^{\rm b}$
Film	$79.48 \pm 1.83^{\mathrm{a}}$	$40.33 \pm 2.51^{\circ}$	$67.43 \pm 2.89^{\mathrm{b}}$
Foam	$0.00\pm0.00$	$0.00\pm0.00$	$0.00\pm0.00$
Fiber	$4.40\pm0.66^{\rm b}$	$0.00\pm0.00^{\circ}$	$11.00\pm1.35^{\rm a}$
More with some concentration within the next one not similar the different $(\mathbf{P} > 0.05)$			

Means with same superscript within the rows are not significantly different (P > 0.05) **Polymeric variations of Microplastics in Fish samples** 

The analysis of polymeric variations across different fish samples (intestine, gill, muscle) and water samples from three aquaculture farms (PF, UF, HF) revealed significant differences in the abundance of various polymers, as presented in Table 5. In the intestine samples, the HF farm had the highest abundance of PET ( $37.00 \pm 3.00$ ), which was significantly greater than UF (27.00  $\pm$  3.00) and PF (0.00  $\pm$  0.00) (P < 0.05). LDPE was most abundant in UF (60.00  $\pm$  3.00), followed by PF (29.33  $\pm$  3.05), with HF showing significantly lower levels (18.00  $\pm$ 2.00). PE was detected only in PF (10.33  $\pm$  1.52), with none found in UF or HF. In the gill samples, PE was highest in HF (70.33  $\pm$  2.51), significantly greater than in PF (56.66  $\pm$  3.51) and UF ( $38.00 \pm 2.00$ ). LDPE levels were comparable between PF ( $31.33 \pm 1.52$ ) and UF (30.33 $\pm$  3.51), with none detected in HF. PET was found exclusively in UF (11.66  $\pm$  2.08), with no presence in PF or HF. For the muscle samples, PE was again most abundant in HF (60.33  $\pm$ 1.52), significantly greater than PF (45.33  $\pm$  2.51) and UF (70.33  $\pm$  3.51). LDPE levels were comparable between UF ( $32.00 \pm 2.00$ ) and PF ( $29.33 \pm 2.08$ ), with none detected in HF. PET was detected only in PF (21.33  $\pm$  1.15), with no presence in UF or HF. In the water samples, PET was most abundant in HF (52.00  $\pm$  2.00), followed by PF (31.66  $\pm$  2.08), with UF showing intermediate levels (23.00  $\pm$  3.00). HDPE was highest in UF (38.00  $\pm$  2.00), with lower values in PF (31.66  $\pm$  1.52) and HF (41.00  $\pm$  1.73). PP content was highest in HF (39.66  $\pm$  1.52), followed by UF (15.00  $\pm$  3.00), while PF showed significantly lower levels (31.33  $\pm$  1.52). Overall, significant differences in the abundance of various polymers were observed across the farms for all tissue types and water samples, indicating potential differences in environmental exposure and farming practices.

Parameters	PF (Farm 1)	UF (Farm 2)	HF (Farm 3)
1 Intestine			
PET	$0.00\pm0.00^{\circ}$	$27.00\pm3.00^{\rm b}$	$37.00\pm3.00^{\rm a}$
HDPE	$24.33\pm2.08^{\rm a}$	$0.00\pm0.00^{\circ}$	$8.66 \pm 1.52^{ m b}$
LDPE	$29.33\pm3.05^{\mathrm{b}}$	$60.00\pm3.00^{\mathrm{a}}$	$18.00\pm2.00^{\circ}$
PP	$33.33 \pm 1.52^{\mathrm{a}}$	$12.00\pm1.73^{\mathrm{b}}$	$32.33\pm2.08^{\rm a}$
PE	$10.33 \pm 1.52^{\mathrm{b}}$	$0.00\pm0.00^{\mathrm{b}}$	$0.00\pm0.00^{\mathrm{b}}$
2 Gill			
PET	$0.00\pm0.00^{\circ}$	$11.66 \pm 2.08^{b}$	$0.00\pm0.00^{\circ}$
HDPE	$0.00\pm0.00^{\circ}$	$0.00\pm0.00$	$0.00\pm0.00^{\circ}$
LDPE	$31.33 \pm 1.52^{\mathrm{a}}$	$30.33\pm3.51^{\mathrm{a}}$	$0.00\pm0.00$
PP	$11.00 \pm 1.00^{\circ}$	$19.66 \pm 1.52^{\mathrm{b}}$	$32.33\pm2.51^{\rm a}$
PE	$56.66 \pm 3.51^{\mathrm{b}}$	$38.00\pm2.00^{\circ}$	$70.33\pm2.51^{\rm a}$
3. Muscle			
PET	$21.33 \pm 1.15^{\rm a}$	$0.00\pm0.00^{\circ}$	$0.00\pm0.00^{ m b}$
HDPE	$0.00\pm0.00^{\rm b}$	$0.00\pm0.00^{\circ}$	$41.00\pm1.73^{\rm a}$
LDPE	$29.33\pm2.08^{\mathrm{b}}$	$32.00\pm2.00^{\mathrm{a}}$	$0.00\pm0.00^{\circ}$
PP	$0.00\pm0.00$	$0.00\pm0.00^{\circ}$	$0.00\pm0.00$
PE	$45.33 \pm 2.51^{\circ}$	$70.33 \pm 3.51^{\mathrm{a}}$	$60.33 \pm 1.52^{b}$
4 Water			
PET	$31.66\pm2.08^{\mathrm{b}}$	$23.00\pm3.00^{\circ}$	$52.00\pm2.00^{\rm a}$
HDPE	$31.66 \pm 1.52^{\mathrm{b}}$	$38.00\pm2.00^{\mathrm{a}}$	$0.00\pm0.00^{\circ}$
LDPE	$10.00 \pm 1.00^{\circ}$	$23.00\pm2.64^{\rm a}$	$8.66 \pm 1.52^{\mathrm{b}}$
PP	$31.33 \pm 1.52^{\mathrm{b}}$	$15.00\pm3.00^{\circ}$	$39.66\pm1.52^{\rm a}$
PE	$0.00\pm0.00$	$0.00\pm0.00$	$0.00\pm0.00$

 Table 5: Polymeric Variations of Microplastics in Fish and Water Samples from

 Different Farms

Means with same superscript within rows are not significantly different (P > 0.05)

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### Physico-chemical parameters of Fish farm

The analysis of physico-chemical parameters of water samples from three aquaculture farms (PF, UF, HF) revealed significant differences in various water quality indicators, as presented in Figure 6 below.

In terms of temperature, the HF farm had the highest value ( $28.06 \pm 0.12$ ), which was significantly greater than UF ( $27.04 \pm 0.04$ ) but comparable to PF ( $27.53 \pm 0.45$ ). The pH levels were similar across the farms, with HF showing the highest value ( $7.67 \pm 0.24$ ), followed by UF ( $7.46 \pm 0.39$ ) and PF ( $7.38 \pm 0.20$ ), with no significant differences (P > 0.05). For biological oxygen demand (BOD), PF ( $4.93 \pm 0.11$ ) and UF ( $5.12 \pm 0.23$ ) exhibited similar levels, while HF had a significantly lower value ( $3.84 \pm 0.20$ ) (P < 0.05). Dissolved oxygen (DO) was highest in HF ( $10.86 \pm 0.19$ ) and PF ( $10.65 \pm 0.33$ ), while UF showed a significantly lower value ( $8.63 \pm 0.09$ ).

The chemical oxygen demand (COD) levels were consistent across all farms, with PF (11.66  $\pm$  0.24), UF (11.63  $\pm$  0.17), and HF (11.64  $\pm$  0.36) showing no significant differences (P > 0.05). Ammonia concentrations were also similar across the farms, with UF having the highest value (0.62  $\pm$  0.04), followed by HF (0.56  $\pm$  0.13) and PF (0.44  $\pm$  0.11), with no significant differences. Phosphate levels were significantly higher in HF (15.58  $\pm$  0.50), compared to UF (9.43  $\pm$  0.33) and PF (8.48  $\pm$  0.24) (P < 0.05). Similarly, nitrite was highest in HF (5.53  $\pm$  0.23), significantly greater than UF (2.27  $\pm$  0.32) and PF (2.48  $\pm$  0.25) (P < 0.05). Nitrate levels, however, were comparable across farms, with PF (0.62  $\pm$  0.17), UF (0.33  $\pm$  0.06), and HF (0.66  $\pm$  0.17) showing no significant differences. Overall, significant differences in water quality parameters such as BOD, DO, phosphate, and nitrite were observed across the farms, suggesting potential variations in environmental conditions and water management practices.

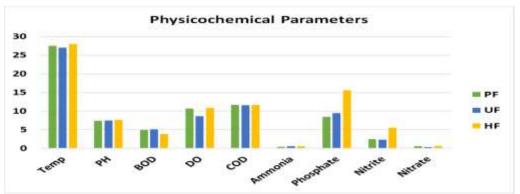


Fig 5: Physico chemical parameters of the water samples in Different Farms

### DISCUSSION

The Results of this study provide valuable insights into the distribution and abundance of microplastics across various fish tissues and their aquatic environment, with significant variations observed between fish farms and tissue types. The presence of microplastics in both the fish and water samples reflects the potential environmental contamination at the farms, and the results indicate tissue-specific accumulation patterns.

The results in Table 1 reveal significant differences in microplastic concentrations across the three fish farms. The highest microplastic concentration in gills was observed in fish from UF,

while the lowest was in PF. This may be as a result of differences in water quality management practices, feed types or proximity to sources of plastic pollution. The gills, being the primary respiratory organ in fish, are in constant contact with the water, making them particularly susceptible to microplastic exposure [33]. The relatively higher accumulation in UF indicates increased microplastic presence in the farm's water, potentially linked to its feed source or surrounding environment.

In the intestines, the highest concentration of microplastics was observed in HF, which may be attributed to the ingestion of contaminated feed or direct water exposure. The presence of microplastics in the intestines aligns with previous studies that report the ingestion of plastic particles by fish, which then accumulate in the gastrointestinal tract [34]. On the other hand, the muscle tissue, which represents the edible portion of the fish, showed lower levels of microplastics compared to the gills and intestines. However, the muscle from HF had the highest concentration, raising concerns about food safety for consumers. The muscle tissue is typically less exposed to environmental contaminants compared to the gills and intestines, which might explain the lower levels [35].Water samples followed a similar trend, with HF exhibiting the highest microplastic abundance. The high contamination levels in the water samples from HF could explain the significant accumulation of microplastics in the fish tissues from this farm. The water acts as a major reservoir for microplastics, which are then absorbed or ingested by aquatic organisms, leading to bioaccumulation in their tissues. This finding underscores the importance of monitoring water quality in aquaculture systems to mitigate potential health risks associated with microplastic exposure in both fish and humans [36].

Comparatively, the intestine had the highest concentration of microplastics, significantly more than the gills and muscle. This could be due to the ingestion of microplastic particles, which are then trapped in the intestinal lining. The gills also exhibited considerable microplastic contamination, which can be explained by the continuous filtration of water during respiration. The muscle showed the lowest levels of microplastic contamination, likely due to lower direct exposure to contaminated water or food, as well as slower translocation of particles from the digestive system to the muscle tissue. The results is in agreement with previous research on microplastic contamination in fish, suggesting that exposure to contaminated water and ingestion of microplastics from the environment or feed are the primary pathways for microplastic accumulation in fish tissues [37]. The differential accumulation across tissues indicates that the route of exposure and tissue-specific uptake mechanisms play critical roles in determining microplastic levels in different parts of the fish. The high levels of microplastics in the water samples, especially from HF, suggest that the environmental contamination levels directly influence the degree of microplastic accumulation in the fish[38]. The length of microplastics is an important factor in determining their potential impacts on fish physiology and the possible risks they pose to consumers through seafood ingestion. The variation in microplastic length across different farms and tissues suggests that multiple factors, such as water quality, feed contamination, and environmental management practices, contribute to the accumulation of microplastics in fish. The longer microplastic particles found in water and tissues reveals the potential risks posed by larger microplastics, which may cause physical damage to fish and negatively affect their growth and survival [39].

The results of this study reveal that the HF exhibited the highest average lengths of microplastics (MPs) in both fish tissues and water samples, suggesting significant contamination within this aquaculture system. Specifically, the average lengths of MPs recorded were  $814.34 \mu m$  in gills,  $1161.33 \mu m$  in intestines,  $806.00 \mu m$  in muscle, and 1692.00

µm in water. These results raise critical concerns regarding the environmental health of the HF farm and the potential risks associated with the consumption of fish from this location. One potential explanation for the increased lengths of MPs in the HF farm may be related to the specific practices associated with intensive aquaculture systems. In intensive farming, highdensity stocking and the use of artificial feeds can contribute to the accumulation of larger MPs in the aquatic environment [40]. Fish feeds often contain additives and fillers, some of which may include microplastics originating from packaging or the feed manufacturing process [41]. If the feed used in HF is derived from sources that incorporate plastic materials or is inadequately processed, this could lead to higher lengths of microplastic contamination in the fish tissues. It is well-documented that aquaculture fish can ingest microplastics either through their diet or directly from contaminated water, leading to significant health risks [41]. This is particularly concerning given the long lengths of MPs found, as larger particles pose greater risks for physical obstruction and toxicological effects on fish.Additionally, the handling and operational practices at the HF farm may play a significant role in microplastic contamination. For instance, the use of plastic materials for equipment, such as nets, feeding apparatus, and tanks, can inadvertently introduce microplastics into the system. Abrasion and wear of these materials during normal operations may result in the release of microplastic particles into the water, which fish then ingest, leading to the longer lengths observed [42].

Furthermore, the observed length variations in MPs suggest that the filtration and waste management practices at HF may be insufficient. The presence of larger MPs in both the aquatic environment and fish tissues indicates a potential failure in the farm's waste management systems to adequately filter out larger plastic particles. Research has indicated that inefficient filtration systems can lead to elevated levels of MPs in fish, ultimately affecting their growth, health, and overall welfare [43]. The elevated average lengths of MPs in the HF farm indicates urgent need for improved aquaculture management practices, including the implementation of effective water filtration systems and the monitoring of feed quality. These measures can be effective in mitigating the environmental and health impacts of microplastic contamination, ensuring the safety of fish products for consumers and preserving aquatic ecosystem integrity [44]. The color variation of microplastics in samples from the fish farm suggests insights into their sources, degradation processes, and environmental interactions, indicating that brightly colored microplastics may originate from consumer products and reflect local pollution, while faded colors may indicate aging due to environmental exposure and these variations can influence ingestion rates by aquatic organisms and potentially affect food web dynamics [45]. The dominance of white microplastics in the intestine, gill and water samples has become a significant concern in recent studies, indicating specific sources and potential ecological impacts. White microplastics are commonly derived from a variety of consumer products, including packaging materials, polystyrene, and synthetic fibers. Their prevalence in ecosystems suggests that these materials are widely used and often inadequately disposed of, leading to increased plastic pollution in aquatic environments [46].

Research has shown that white microplastics may be more prominent due to their production processes and the applications of the polymers used. For instance, polystyrene, often used in food containers and packaging, is frequently produced in white or translucent colors [47]. As a result, when these materials break down in the environment, they contribute significantly to the abundance of white microplastics found in aquatic habitats.

The persistence of white microplastics can also be attributed to their resistance to environmental degradation. Unlike colored plastics, which may fade due to UV exposure, white

microplastics tend to retain their color longer, increasing their likelihood of accumulation in sediments and marine organisms [48]. This accumulation poses risks to aquatic life, as microplastics can be mistaken for food by fish and other marine organisms, potentially leading to bioaccumulation and associated toxicological effects [49].

The results in Table 4 reveal significant differences in the morphological variations of microplastics across the three fish farms. The intestines exhibited the highest abundance of filament microplastics in HF, while the lowest was in PF. This variation in filament microplastic abundance across farms suggests that water quality, feed contamination, and farm management practices may be contributing factors. Filament microplastics, commonly associated with synthetic fibers from textiles, are ingested by fish through contaminated water or feed, leading to their accumulation in the digestive tract [50]. The high filament count in HF points to greater environmental exposure, possibly linked to proximity to industrial activities or agricultural runoff. Pellet microplastics were most abundant in PF, which may indicate exposure to plastic waste, such as bottle caps or packaging materials, that degrades into smaller particles. This is in line with previous studies showing that microplastics from consumer products often enter aquatic environments and accumulate in fish tissues [51]. Foam particles, however, were predominantly found in UF, which may suggest contamination from lightweight plastics, possibly from packaging or insulation materials that float in water and persist in the environment. The gills, being in constant contact with water, also showed significant morphological variations. UF had the highest concentration of filament microplastics in the gills, while no filaments were found in PF or HF. This may be due to differences in water quality management or the proximity to sources of plastic pollution [52]. Foam particles were found only in PF, suggesting a unique environmental source or a specific type of pollution affecting this farm's water supply. In muscle tissues, UF exhibited the highest abundance of filament microplastics. This finding raises concerns about the retention of long microplastic particles in fish tissues, which can lead to health risks for consumers if ingested. Filament microplastics are known for their ability to persist in tissues, making them more likely to accumulate over time. Film microplastics were most prevalent in HF muscle, potentially due to the translocation of flexible plastic particles from water or feed to edible tissues, posing a risk to food safety [53].

Water samples followed a similar pattern, with UF showing the highest pellet concentrations and HF having the most film microplastics. These findings highlight the importance of water quality monitoring and the role of environmental contamination in determining the types of microplastics that accumulate in fish tissues (Wong *et al.*, 2020). The abundance of film microplastics in HF water suggests ongoing plastic fragmentation in the environment, contributing to contamination in both water and fish tissues [54]. The findings align with previous studies on microplastic contamination in aquaculture, indicating that external environmental factors, such as water quality and feed sources, play a critical role in the accumulation of microplastics in fish tissues [55]. These results emphasize the need for effective waste management and filtration systems to mitigate the risks associated with microplastic exposure in both fish and humans.

For the polymeric variations, PET was found in the highest concentration in the intestines of fish from HF, while it was absent in PF. This difference may be due to the proximity of HF to sources of plastic pollution, as PET is commonly found in packaging materials like bottles and containers, which degrade into microplastics in aquatic environments [56]. The high presence of PET in HF could indicate contamination through water or feed, raising concerns about

environmental exposure to plastic waste. HDPE was detected only in HF intestines, possibly due to the use of this polymer in items such as plastic bottles, containers, and pipes. This finding is consistent with research showing that HDPE degrades into microplastic particles through physical stress and chemical reactions in aquatic environments (Khan et al., 2023). The absence of HDPE in PF and UF suggests that the water sources in these farms may have lower contamination from this polymer, potentially due to better waste management practices [57].LDPE, commonly used in bags and packaging, showed the highest abundance in UF intestines, followed by PF. The presence of LDPE in both farms suggests that it is likely ingested through contaminated water or feed, consistent with reports that LDPE is highly prone to fragmentation and is a significant contributor to microplastic contamination in aquaculture environments [58]. PP was present in all farms, but HF showed the highest levels in gills, indicating potential exposure through the water. PP is used in a variety of products, from packaging to textiles, and can degrade into microplastics over time. The high PP concentration in HF could be related to industrial activities or agricultural runoff near the farm [59].PE, the most widely produced plastic globally, was highly concentrated in HF gills and muscle tissues, with lower levels detected in UF and PF. PE's ubiquitous use in packaging and its resistance to degradation make it a common pollutant in aquatic environments. The significant accumulation of PE in HF suggests that this farm may be experiencing higher levels of plastic contamination, potentially from surrounding industrial or urban activities. These findings align with previous studies that highlight the role of environmental contamination and farming practices in determining the polymeric composition of microplastics in fish tissues. The significant differences in polymeric variations across farms suggest that factors such as water quality, feed sources, and proximity to pollution sources play a critical role in the accumulation of different types of plastics in aquaculture systems [60].

For the physicochemical parameters, Temperature can influence both the behavior of microplastics and the metabolism of aquatic organisms. Elevated temperatures in HF might suggest a more contaminated or polluted water source, where increased microbial activity could potentially influence the breakdown of plastics into microplastic particles [61]. Additionally, temperature changes can impact the solubility and transport of pollutants, potentially affecting the bioavailability of microplastics and other contaminants [62]. The pH levels range from 7.38 in PF to 7.67 in HF, indicating slightly alkaline conditions in all water samples. pH can influence the degradation of plastics in water, as certain materials break down more easily under acidic or basic conditions. In neutral to slightly alkaline waters, plastics like polyethylene (PE) and polypropylene (PP) tend to be more stable, meaning they persist longer without breaking down into microplastics [63]. The small increase in pH in the HF group could be associated with increased pollution, potentially affecting plastic degradation processes. BOD levels are highest in UF and lowest in HF, suggesting that the HF group may have less organic pollution or greater microbial activity consuming oxygen. A lower BOD might also correlate with higher levels of contaminants such as microplastics, as lower BOD could mean less organic matter is available for microbes to break down [64]. Elevated BOD in UF could indicate higher organic matter, which could influence microplastic interactions and absorption of pollutants. Dissolved oxygen levels vary significantly across the groups, with UF having the lowest DO, while both PF and HF have higher values. DO is crucial for the survival of aquatic organisms, and lower levels in the UF group might suggest higher organic pollution, which could impact fish health and increase the ingestion of microplastics [65].

Additionally, lower DO levels may facilitate anaerobic conditions that could affect the degradation of certain plastic types, making them more persistent in the environment. COD is relatively consistent across the treatments, with values around 11.63 mg/L. COD measures the amount of oxygen required to chemically oxidize organic and inorganic compounds in water. The stable COD levels suggest that the presence of microplastics may not be significantly influencing the chemical oxidation processes in these water samples [66]. However, the presence of microplastics could still interact with other pollutants in the water, possibly affecting overall water quality. Ammonia levels are highest in UF and lowest in PF. Elevated ammonia in the UF group might suggest greater levels of organic pollution or waste, which can affect the health of aquatic organisms and the environment [67]. Ammonia is also a key nutrient that can influence the growth of microbial communities, potentially accelerating the breakdown of organic material and impacting the interaction of microplastics with the surrounding water matrix [68]. Phosphate levels are highest in HF indicating significant nutrient pollution, which could lead to eutrophication. Elevated phosphate levels may also influence the behavior of microplastics in water, as they can adsorb onto plastic surfaces, potentially increasing the toxicological risks associated with microplastic ingestion by aquatic organisms [69]. Eutrophication can exacerbate the accumulation of plastics in aquatic environments by altering the flow and settling patterns of particles. Nitrite and nitrate levels show variation across the groups, with the highest nitrite levels in HF and highest nitrate levels in PF. Elevated nitrite levels in HF suggest more contamination or nutrient loading, which can affect water quality and fish health. Nitrite and nitrate are indicators of nutrient pollution, often from agricultural runoff, and can impact the behavior of microplastics by changing the water's chemical composition [70].

### CONCLUSION

This study provides clear evidence of widespread microplastic contamination in fish and water samples from aquaculture farms within Awka Metropolis. The detection of various types of microplastics, such as PET, HDPE, and LDPE, in fish tissues confirms that microplastics are entering aquatic environments through multiple pathways, including industrial and domestic waste. The presence of these particles in the gills and intestines of fish suggests that both ingestion and respiration are significant exposure routes. Importantly, microplastics were also detected in the muscle tissues, which are consumed by humans, raising concerns about food safety and potential health risks. This study underscores the urgent need to address the sources of plastic pollution, particularly in regions with poorly managed waste disposal systems. The growing accumulation of plastics in aquatic environments represents a serious threat to biodiversity, food security, and public health.

### RECOMMENDATIONS

Studies has shown that sources of microplastic pollution in aquaculture farms can be from Fish feeds, water source or improper waste disposal. Therefore, monitoring of fish feed sources and strengthening waste management systems in Awka Metropolis is critical. This should involve the establishment of more efficient recycling programs, including community-wide recycling initiatives that encourage the sorting and recycling of plastic waste at the source. Raising public awareness about the dangers of plastic pollution and the steps individuals can take to mitigate its impact is essential. Educational campaigns should focus on informing residents about how single-use plastics contribute to environmental degradation and the importance of reducing plastic usage. Furthermore, stricter regulations on industries that contribute significantly to plastic pollution, such as packaging, manufacturing, and fishing, should be enforced. Such

measures can include requiring companies to adopt more sustainable packaging options or introducing penalties for improper waste disposal. There should be increased support for research initiatives that explore innovative ways to reduce plastic

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